(P/T) junction have a significant impact on the dynamics of PolBI, indicating the translocation along the single-stranded DNA as the mechanism for the spontaneous motion revealed by FRET. In addition, we have found PCNA not only improves the binding affinity of its cognate polymerase, but suppresses the frequent movement of PolBI from the P/T junction. In summary, many of the dynamics discussed here are reported for the first time and will provide a new perspective for understanding the orchestration of replication-related processes in archaea.

339-Pos

The Dynamic DNA Damage Inducible Protein UmuD Inhibits Replication

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All organisms experience DNA damage from myriad sources. When bacterial cells experience DNA damage, the SOS response is induced, leading to upregulation of at least 57 genes in *E. coli*. The SOS regulated genes include those involved in DNA repair and cell cycle regulation. Also induced as part of the SOS response are Y family DNA polymerases, which have the specialized ability to copy damaged DNA. This specialized ability comes at a potentially mutagenic cost as Y family DNA polymerases replicate undamaged DNA in an error-prone manner. Multiple layers of regulation control the activity of these potentially mutagenic Y family polymerases. UmuD, a small manager protein, and its cleaved form, UmuD', directly interact with both Y family polymerases as well as the beta processivity clamp and the replicative DNA polymerase. We find that UmuD, but not UmuD′, inhibits primer extension by the DNA polymerase III alpha subunit. We probed the conformation and dynamics of the *umuD* gene products. Thermal shift experiments show that UmuD undergoes two melting transitions, one likely due to the dissociation of the N-terminal arms and the other due to unfolding of the globular domain. We used hydrogen-deuterium exchange mass spectrometry (HXMS) to probe the conformations of UmuD and UmuD'. In HXMS, backbone amide hydrogens become labeled with deuterium over time. Our HXMS results reveal that the N-terminal arm of UmuD, which is not present in the cleaved form UmuD', is highly dynamic. Residues that are likely to contact the N-terminal arm show more protection from exchange in UmuD than UmuD′. Additionally, there are regions of both proteins that are less dynamic. Our observations are consistent with the proposed model of UmuD and the finding that UmuD is relatively unstructured.

340-Pos

A Three Pool Model of DNA Digest Gels

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The purpose of the project is to determine the effectiveness and to clarify the mechanism of action of potential antibiotic compounds to interfere with the mechanisms of DNA repair in bacteria. After treating the bacteria with the compounds of interest (norfloxacin, novobiocin, and a novel antibiotic, peptide wrwycr), the DNA, now broken into many fragments, was separated based on size using pulse field gel electrophoresis. Preliminary analysis of the gels reveals three pools of DNA fragments: (1) unbroken, (2) broken at a few random spots into fragments larger than about 30 kb and described by a Poisson distribution, and (3) digested into fragments smaller than 30 kb, probably with help of the exonuclease RecBCD. Fits to these three pools are presented and the implications for antibiotic activity are discussed.

341-Pos

Direct Visualization of Fluorescent SSB on Single Molecules of ssDNA as a Mechanistic Probe in the Early Stages of Homologous Recombination

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In all organisms homologous recombination (HR) is essential for the efficient and error-free repair of DNA lesions. Defects in HR result in genomic instability, which often manifests in humans as a genetic disposition to cancer. Central to the process of homologous recombination is the strand exchange activity of the RecA/Rad51 class of proteins. Through the formation of a pre-synaptic filament on single stranded DNA (ssDNA), RecA/Rad51 aligns a broken chromosome with an intact one (a process called synapsis), allowing for a subsequent array of potential repair pathways. During the presynaptic stage, filament formation is inhibited by the diffusion-limited association of ssDNA with the high-affinity single stranded DNA binding protein, SSB/RPA. A class of positive regulators called mediators facilitate filament formation by alleviating this biochemical inhibition. These mediators include RecF/O/R (E. coli), Rad52 (S. cerevisiae and H. sapiens), and BRCA2 (H. sapiens). We have fluorescently modified several of the key proteins involved in pre-synaptic filament formation in E. coli, specifically SSB and RecA. Here we present their characterization and utility as fluorescent biochemical sensors for single-stranded DNA in single molecule assays designed to mechanistically probe the early, pre-synaptic stage of homologous recombination.

342-Pos

Analysis of Dynamic Properties of DNA Repair Protein MutS and DNA Complexes Using Molecular Dynamics Simulations

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DNA mismatch repair (MMR) maintains genome stability by repairing mismatches that arise through DNA replication errors and during recombination. Defects in MMR result in a significant increase in the spontaneous mutation rate and predispose humans to cancer.

In *E. coli*, the proteins MutS, MutL and MutH are responsible for the MMR. MMR is initiated by MutS, which functions in the homodimer form. MutS recognizes and efficiently binds to mispaired bases and unpaired bases in DNA duplexes. It is thought that the ATPase activity of MutS plays a role in proofreading to verify mismatch binding and authorize the following downstream excision in which MutL and MutS are involved.

However, little is known of the relationship between the recognition of DNA and the ATP hydrolysis by MutS at the atomic level. In order to investigate how the binding of MutS to the DNA and ATP hydrolysis are coordinated, molecular dynamics (MD) simulations of the wild-type and mutant MutS in water with mismatched and undamaged DNA were performed. Including the water molecules, each system comprised about 200,000 atoms. The MD simulations were carried out at a constant pressure of one bar and a temperature of 300 K for several tens of nanoseconds in total. The binding free energies were calculated using the MM-GBSA method.

It was found that the interaction between MutS and DNA changes significantly according to the different kinds of mismatch base pair or different kinds of mutation in MutS. It was shown that the electrostatic energy significantly contributed to the binding free energies. Moreover, a correlation between the binding free energies and the functional movement of MutS was observed.

343-Pos

Base Pair-Position-Specific DNA 'Breathing' At the Replication Fork Junction Regulates Helicase Access

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Thermal fluctuations induce transient opening of base pairs in dsDNA constructs. In previous studies with DNA constructs of conserved sequence containing 2-AminoPurine (2-AP) probes, we showed that position-specific base-pair (bp) fraying that depends on proximity to the ss/ds junction can be observed in forked DNA constructs of conserved sequence, and that significant (>1%) thermal fraying of base-pairs at helix ends extends 2-3 bps into the dsDNA. Here we build on these results to study the initial steps of DNA helicases at replication forks. Proteins that bind preferentially to ssDNA can capture thermally frayed bps without the expenditure of chemical (NTP-dependent) free energy. The bacteriophage T4 DNA replication complex provides a favorable model system to study basic helicase mechanisms. The T4 helicase-primase (gp41-gp61) sub-assembly forms a tight-binding helicase that unwinds dsDNA and translocates processively along ssDNA lattices, driven by NTP binding and hydrolysis. We use fluorescence and low energy CD spectral signals of site-specifically placed 2-AP probes to monitor the initial steps of helicase activity at a forked DNA construct. We find, on binding a helicase-primase complex to the DNA construct in the presence of non-hydrolysable NTP, that the first bp on the duplex side of the fork opens and additional destabilization penetrates to ~ the 3rd bp. This is consistent with a largely passive mechanism for helicase-dependent DNA unwinding, with the helicase complex binding on the 5′→3′ loading strand at the fork and trapping the first adjacent bp as it is opened by thermal fluctuations.

344-Pos

Single-Molecule Studies of the ssDNA Binding Activity of E. Coli MutL

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MutL stimulates the DNA duplex unwinding activity of UvrD in methyl-directed DNA mismatch repair (MMR) via their physical interactions. However, the molecular functions of MutL associated with the DNA binding and UvrD helicase have been partially understood. We present the kinetic characteristics of the single-stranded DNA (ssDNA) binding activity of MutL in the absence or the presence of UvrD helicases using the single-molecule techniques. The lengthening of the ssDNA due to the ssDNA binding of MutL allows us to observe association and dissociation of MutL from the ssDNA in real-time. In this